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EXAMINER	
HILL, KEVIN KAI	
ART UNIT	**PAPER NUMBER**
1633	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/29/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/642,255	DOLE ET AL.	
	Examiner	Art Unit	
	Kevin K. Hill, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 December 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-41* is/are pending in the application.

4a) Of the above claim(s) *31 and 37* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-30,32-36 and 38-41* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *15 August 2003* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *Dec. 22, 2003, Feb. 14, 2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Effective January 4, 2007, the Art Unit location of your application in the USPTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Kevin K. Hill, Art Unit 1633.

Detailed Action

1. Applicant's response to the Requirement for Restriction, filed on December 12, 2006 is acknowledged.

Applicant has elected the invention of Group II, Claims 1-30, 32-36 and 38-41, drawn to an *in vivo* gene therapy method for treating critical limb ischemia (CLI). Claims 31 and 37 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Within Group I, Applicant has elected the eNOS polypeptide species to comprise a first mutation at a position corresponding to amino acid 495 and a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO:1, as recited in Claim 7.

However, the species election regarding the eNOS polypeptides comprising the first, second and third mutations has been withdrawn because those sequences are free of the prior art. Applicant may access the results of the sequence searches in SCORE.

Within Group I, Applicant has elected the angiogenic factor species "FGF", as recited in Claim 30.

2. Election of Applicant's invention(s) was made with traverse.

Applicant argues that examination of Groups I-III would not pose a significant search burden, as the subject matter is expected to yield significantly overlapping results. Applicant also argues that methods of treating a condition with a polynucleotide or a polypeptide would be co-extensive.

Applicant's species elections were made with traverse. Applicant argues that although each angiogenic factor is patentably distinct and independent, the factors share a common functionality, specifically affecting angiogenesis, and thus all should be examined.

Applicant's arguments have been fully considered but are not found persuasive. MPEP §803 states that "If the search and examination of all the claims in an application can be made without serious burden, the Examiner must examine them on the merits, even though they include claims to independent or distinct inventions."

In the instant case a serious burden exists since each limitation, directed to *in vivo* and *ex vivo* treatment methods comprising the administration of a polynucleotide or a polypeptide, requires a separate, divergent, and non co-extensive search and examination of the patent and non-patent literature. For instance, a search and consideration of the prior art as it relates to treating a condition comprising the *in vivo* administration of a polynucleotide would not be adequate to uncover prior art related to treating a condition comprising the *in vivo* administration of a polypeptide. With respect to the Markush Group of angiogenic factors, each factor, despite Applicant's argument, has distinctly different biological activities, signals through distinctly different receptors, and causes distinctly different effects that are non-identical and non-obvious. Applicant has not provided evidence to the contrary.

Further, a search and examination of all the claims directed to the recited embodiments involves different considerations of novelty, obviousness, written description, and enablement for each claim. In view of these requirements, it is the Examiner's position that searching and examining all of the claims including limitations to a method of treating critical limb ischemia comprising the *in vivo* administration of a polypeptide in the same application presents a serious burden on the Examiner for the reasons given above and in the previous Restriction Requirement.

The requirement is still deemed proper and is therefore made FINAL.

3. Claims 31 and 37 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

4. Claims 1-30, 32-36 and 38-41 are under consideration.

Priority

5. Applicant's claim for the benefit of a prior-filed application, parent provisional application 60/403,637, filed on August 16, 2003 under 35 U.S.C. 119(e) is acknowledged. Accordingly, the effective priority date of the instant application is granted as August 16, 2003.

Information Disclosure Statement

Applicant has filed Information Disclosure Statements on December 22, 2003 and February 14, 2005. These have been considered. The signed and initialed PTO Forms 1449 are mailed with this action.

Drawings

New corrected drawings in compliance with 37 CFR 1.121(d) are required in this application because the data appear to be incorrectly graphed. The disclosure regarding what parameters are being measured, exactly what and how the ratio(s) are established, and how those ratio(s) values are charted in the figures is unclear (pgs 36-37). The data, as presented in Figures 4, 8, 14, 20 and 25, chart "Ischemia/Normal blood flow ratio", thus indicating that the degree of ischemia is graphed as compared to normal blood flow. As the denominator value "normal blood flow" increases, the numerical value of the Ischemia/Normal blood flow ratio (as indicated on the ordinate) will decrease. Similarly, as the numerator value of "ischemia" decreases, the numerical value of the Ischemia/Normal blood flow ratio will decrease. The graphs presently indicate that the animals treated with the inventive polynucleotide actually experience greater ischemia than the non-treated controls.

Furthermore, Figure 25 (page 25/28) is entitled as "Figure 7"; however, the title "Figure 7" is originally used in the figure on page 7/28. Applicant is strongly encouraged to review each figure for correctness in charting, graphing, data presentation and proper titles.

Applicant is advised to employ the services of a competent patent draftsperson outside the Office, as the U.S. Patent and Trademark Office no longer prepares new drawings. The corrected drawings are required in reply to the Office action to avoid abandonment of the application. The requirement for corrected drawings will not be held in abeyance.

Claim Objections

6. **Claims 1 and 35-36, and claims dependent therefrom, are objected to because of the following informalities**:

These claims each identify eNOS as a polypeptide that may be used in the claimed invention. However, the claims do not first identify the polypeptide by its complete name prior to using its acronym. The abbreviation should be spelled out in the first appearance of the claims and should be followed by the abbreviation in parentheses, e.g. critical limb ischemia (CLI).

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. **Claims 1-30, 32-36 and 38-41 are rejected under 35 U.S.C. 112, first paragraph**, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Vas-cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states that Applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was

in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." (See page 1117.) The specification does not clearly allow persons of ordinary skill in the art to recognize that (he or she) invented what is claimed." (See *Vas-cath* at page 1116).

The claimed invention is directed to a method comprising the administration of a nucleic acid functionally encoding an endothelial nitrogen oxide synthase (eNOS) protein or a mutant thereof. Furthermore, Claim 20 is drawn to a polynucleotide encoding an eNOS polypeptide mutant, wherein the amino acid sequence of said polypeptide mutant is substantially homologous to the amino acid sequence of the eNOS polypeptide mutant of Claim 4, and Claim 21 is drawn to an isolated eNOS polypeptide mutant having 95-99% sequence identity to the amino acid sequence of said polypeptide mutant of Claim 20.

In analyzing whether the written description requirement is met for genus claims, it is first determined whether a representative number of species have been described by their complete structure. However, the written description in this case only sets forth the human eNOS polypeptide of SEQ ID NO:1 and polypeptide mutants having specific mutations at residues 2, 495 and 1177 of SEQ ID NO:1, therefore the written description is not commensurate in scope with the claims which read on any and all mutants that are substantially homologous to the eNOS mutant of Claim 4.

The specification discloses a subgenus i.e. the eNOS polypeptide mutant having mutation at residue 495 and/or 1177 and 2 of human eNOS, wherein the eNOS polypeptide may comprise a first mutation corresponding to amino acid 495 of SEQ ID NO:1, wherein the first mutation may be any one of 17 amino acid substitutions, but preferably Alanine, Valine, Leucine or Isoleucine (Claim 9), a second mutation corresponding to amino acid 1177 of SEQ ID NO:1, wherein the second mutation is an amino acid substitution to Asp (Claim 10), and a third mutation corresponding to amino acid 2 of SEQ ID NO:1, wherein the third mutation may be Alanine (Claim 11). There is a lack of a written description regarding which amino acids within the full-length amino acid sequence of the eNOS polypeptide of Claim 1 that can be changed by deletion, addition, substitution and combination thereof (pg 14-15, joining ¶) such that the resulting homologs that have alterations in amino acid sequence still have the same function as

the eNOS polypeptide of Claim 1. Applicant does not appear to have reduced to practice any homologs that have alterations in amino acid sequence of the eNOS polypeptide of Claim 1, except for S1177D. Neither has Applicant provided sufficient descriptive information such as definitive structural or functional features that are common to the genus of the eNOS polypeptide mutants. That is, the specification provides neither a representative number of the genus, nor does it provide a descriptive of structural features that are common to the genus. Since the disclosure fails to describe the common attributes or characteristics that identify members of the genus, and because the genus is highly variant, the disclosure of a subgenus (mutants of Claims 9-11) is insufficient to describe a highly variant genus. Because the genus of molecules is extensive and the artisan cannot envision the detailed structure of the encompassed mutations, conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Thus one of skill in the art would not be able to recognize that Applicant was in possession of the invention as now claimed.

The Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶1 "Written Description" Requirement make clear that the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the Applicant was in possession of the genus (Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001, see especially page 1106 3rd column).

Except for disclosing that the nucleic acid encodes an eNOS polypeptide, the specification and claim(s) do not indicate what distinguishing attributes are shared by the members of the genus. The scope of the claims include numerous structural variants, and the genus is highly variant because a significant number of structural differences between genus members is permitted (pg 20, lines 14-21). Although the specification states that these types of changes are routinely done in the art, the specification and claim(s) do not provide any guidance as to what changes should be made. Structural features that could distinguish compounds in the genus from others in the nucleic acid class are missing from the disclosure. No common

structural attributes identify the members of the genus. The general knowledge and level of skill in the art do not supplement the omitted description because specific, not general, guidance is what is needed. Since the disclosure fails to describe the common attributes or characteristics that identify members of the genus, and because the genus is highly variant, the human eNOS of SEQ ID NO:1 and the mutation consisting of the amino acid substitution S1177D of SEQ ID NO:1 alone are insufficient to describe the genus. One of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species to describe the genus.

Consequently, Applicant was not in possession of the instant claimed invention. See *Regents of the University of California v. Eli Lilly and Co.* 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997). Adequate written description of genetic material "'requires a precise definition, such as by structure, formula, chemical name, or physical properties,' not a mere wish or plan for obtaining the claimed chemical invention." Id. 43 USPQ2d at 1404 (quoting *Fiers*, 984 F.2d at 1171, 25 USPQ2d at 1606). The disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter of the claim. Id. 43 USPQ2d at 1406. A description of what the genetic material does, rather than of what it is, does not suffice. Id.

Therefore, only the eNOS mutant having mutations at position 495, 1177 and 2, wherein the first mutation corresponding to amino acid 495 of SEQ ID NO:1 is Alanine, Valine, Leucine or Isoleucine (Claim 9), wherein the second mutation corresponding to amino acid 1177 of SEQ ID NO:1 is Aspartate (Claim 10), and wherein the third mutation corresponding to amino acid 2 of SEQ ID NO:1 is Alanine (Claim 11), but not the full breadth of "eNOS polypeptide mutants" meet the written description provision of 35 U.S.C. §112 first paragraph. Accordingly, this limited information is not deemed sufficient to reasonably convey to one skilled in the art that the Applicant is in possession of the required starting materials, that is the broad genera of nucleic acids encoding the broad genera of eNOS polypeptides, besides the full-length human eNOS of SEQ ID NO:1 and the S1177D variant thereof, to perform the necessary active steps and effect the claimed method at the time the application was filed.

Thus, for the reasons outlined above, it is concluded that Claims 1-30, 32-36 and 38-41 do not meet the requirements for written description under 35 U.S.C. 112, first paragraph.

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

8. **Claims 1-30, 32-36 and 38-41 are rejected under 35 U.S.C. 112, first paragraph,** because the specification, while being enabling for a method of treating critical limb ischemia (CLI) comprising administering by intramuscular injection to a patient in need of treatment an effective amount of a recombinant adenovirus comprising a polynucleotide encoding a mutant mammalian endothelial nitrogen oxide synthase (eNOS) polypeptide, wherein the mutation consists of the amino acid residue corresponding to amino acid residue 1177 of SEQ ID NO:1 being substituted to Aspartate,

does not reasonably provide enablement for an enormous genus of methods of treating CLI comprising an enormous genus of means of administration to a patient of an enormous genus of polynucleotides encoding an enormous genus of normal and mutant mammalian eNOS polypeptides.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention. If not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2ds 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not

disclosed in the specification. Therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention. And thus, skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The Breadth of the Claims and The Nature of the Invention

The breadth of the claims is exceptionally large for encompassing an enormous genus of recombinant vectors and delivery vehicles comprising an enormous genus of polynucleotides encoding an enormous genus of eNOS polypeptides for use in treating CLI. The breadth is further expanded because the invention also recites the administration of a large genus of angiogenic protein factors (Claim 30) in combination with the inventive polynucleotide.

The claims are drawn to a method of treating critical limb ischemia (CLI) comprising the administration of a polynucleotide encoding a human endothelial nitrogen oxide synthase (eNOS) polypeptide, wherein the polypeptide may be wildtype or mutant having mutations in an amino acid sequence corresponding to a functional or regulatory domain of a mammalian eNOS, wherein at least one of said mutations is: i) at a position corresponding to amino acid residue, Thr495, in a calmodulin-binding domain that is phosphorylated in mammalian cells, ii) at a position corresponding to amino acid residue, Ser1177, that is functionally important for nitrogen oxide release and is phosphorylated by the Akt protein serine/threonine kinase; iii) at a position corresponding to amino acid residue Gly2, and iv) any and all eNOS polypeptide mutants wherein the amino acid sequence of said polypeptide mutants is substantially homologous to the amino acid sequence of the eNOS polypeptide mutant of Claim 1.

The inventive concept in the instant application is the administration of a human eNOS polypeptide to promote angiogenesis and effectively treat CLI. Applicant contemplates the eNOS polypeptide to be either wildtype or to contain mutations at amino acid residues important for the regulated activity of eNOS, specifically Thr495, Ser1177 and Gly2. Applicant contemplates that these mutations in human eNOS result in i) an increase or decrease of the eNOS polypeptide (Claim 12), ii) an increased binding affinity for calmodulin of the mutant eNOS polypeptide (Claim 13), iii) a decrease in Ca2+-calmodulin dependent stimulation of the mutant eNOS polypeptide (Claim 14), and iv) an increased eNOS activity, as measured by the generation of nitric oxide (Claims 15-16) and reductase activity (Claims 15, 17). Applicant further

contemplates the targeted delivery of the inventive polynucleotide to endothelial cells (Claim 27) and bone marrow-derived cells (Claim 28) to modulate eNOS activity in these cells, as well as the administration of an angiogenic factor before, during or after the administration of the inventive eNOS polynucleotide, and thus the invention reasonably embraces the arts of gene and protein therapy.

The State of the Prior Art, The Level of One of Ordinary Skill and The Level of Predictability in the Art

Applicant contemplates that the recombinant nucleic acid composition may be introduced *in vivo*, and thus Applicant's invention falls within the realm of gene therapy, which is in the nature of transforming cells with nucleic acids encoding therapeutic molecules to produce a therapeutic effect. Applicant contemplates that the recombinant nucleic acid composition may be introduced by gene delivery methods encompassing various means of *in situ* injection and composition formulations (pgs 25-27). Applicant also comtemplates the use of an enormous genus of viral vectors (pg 23), including retroviruses, adenoviruses and alpha viruses. However, the art recognizes that these viruses are not identical, have different host cell-targeting capabilities, different *cis*-acting regulatory elements that control the expression of the therapeutic polynucleotide, and different capabilities towards induction of host immune responses. Importantly, in contrast to retroviruses, adenoviruses do not integrate their genetic material into the host genome, thus preventing the risk of insertion mutagenesis. It is noted that Applicant contemplates the targeted delivery of the inventive polynucleotide to endothelial and bone marrow-derived cells (Claims 27 and 28, respectively). Thus, not all vectors and delivery vehicles contemplated by Applicant is capable of targeting the recited cell types. Furthermore, as will be discussed below, Applicant only discloses working examples of the invention using intra-muscular injection (pg 35, lines 14-15; pg 36, lines 14-15; pg 37, lines 21-32).

One of ordinary skill in the art recognizes that intra-muscular injections do not reasonably extrapolate to cell-specific targeting of endothelial and/or bone marrow-derived cells because an artisan must consider non-obvious method steps to optimally deliver a therapeutically effective amount of the recombinant nucleic acid to the chosen cell or tissue type, specifically endothelial and/or bone marrow-derived cells, wherein each chosen cell type demands distinctly

different, non-identical cell biological and physiological considerations, e.g. route of administration, to achieve the necessary incorporation of the recombinant nucleic acid and effect the desired therapeutic result. Furthermore, the art recognizes that numerous and diverse cell types exist within, and are derived from, the generically recited "bone marrow-derived" cells including, but not limited to, white blood cells such as lymphocytes, monocytes and neutrophils, hematopoietic stem cells, red blood cells, and mesenchymal stem cells that have the capacity to differentiate into osteoblasts, chondrocytes and myocytes (en.wikipedia.org/wiki/Bone_marrow; last visited January 5, 2007). The specification fails to disclose the necessary guidance so that an artisan may perform the claimed method and target the genus of cell types encompassed by the claims.

With regard to gene therapy, at the effective filing date of the present application, August 16, 2002, the attainment of any therapeutic effect via gene therapy was, and remains, highly unpredictable, let alone for the attainment of prophylactic effects via gene therapy mechanisms as contemplated by Applicants. While progress has been made in recent years for gene transfer *in vivo*, vector targeting to desired tissues *in vivo* continues to be a difficulty as supported by numerous teachings available in the art. There are several known factors that limit an effective human gene therapy, including sub-optimal vectors, the lack of a stable *in vivo* transgene expression, the adverse host immunological responses to the delivered vectors and most importantly an efficient gene delivery to target tissues or cells. For example, Deonarain (Deonarain, M., Expert Opin. Ther. Pat. 8: 53-69, 1998) indicates that:

> "[O]ne of the biggest problems hampering successful gene therapy is the ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time" (page 53, first paragraph).

Deonarain reviews new techniques under experimentation in the art which show promise but states that such techniques are even less efficient than viral gene delivery (page 65, CONCLUSION). Verma and Somia (Nature 389: 239-242, 1997) review vectors known in the art for use in gene therapy and discusses problems associated with each type of vector. The teachings of Verma indicate a resolution to vector targeting has not been achieved in the art

(entire article). Verma also teaches appropriate regulatory elements may improve expression, but it is unpredictable what tissues such regulatory elements target (page 240, sentence bridging columns 2 and 3). Verma states that:

> "the Achilles heel of gene therapy is gene delivery and this is the aspect we will concentrate on here. Thus far, the problem has been an inability to deliver genes efficiently and to obtain sustained expression . . ."

The use of viruses (viral vectors) is a powerful technique, because many of them have evolved a specific machinery to deliver DNA to cells. However, humans have an immune system to fight off the virus, and our attempts to deliver genes in viral vectors have been confronted by these host responses (e.g., p. 239, col. 3). Even in 2005, Verma and Weitzman (Annu. Rev. Biochem. 74:711-738, 2005) still state:

> "The young field of gene therapy promises major medical progress toward the cure of a broad spectrum of human diseases, ranging from immunological disorders to head disease and cancer. It has, therefore, generated great hopes and great hypes, but it has yet to deliver its promised potential", and "[I]f scientists from many different disciplines participate and pull together as a team to tackle the obstacles, gene therapy will be added to our medicinal armada and the ever- expanding arsenal of new therapeutic modalities." (page 732, top of third paragraph).

Goncalves (BioEssays 27:506-517, 2005) also states:

> "Overall, one can conclude that further improvements in gene transfer technologies (e.g. control over transgene expression and integration) and deeper insights in host-vector interactions (e.g. knowledge on vector and gene-modified cell biodistribution following different routes of administration and the impact on innate and adaptive immunity) are warranted before clinical gene therapy reaches maturity" (page 514, right-hand column, last paragraph).

Gardlik et al (Med. Sci. Monit. 11:RA110-121, 2005) conclude:

"Although clinical trials have already started, there are still numerous limitations that must be solved before routine clinical use. Nevertheless, it can be expected that future research will bring tissue- and disease-specific delivery strategies and that this hurdle will be overcome at last" (page RA119, right-hand column, last paragraph).

The art of gene therapy is a high art. Vector based and non-vector based means of introducing DNA into cells for expression have not successfully overcome obstacles related to efficiency of gene delivery and toxicity. Thus, the art recognizes significant obstacles and unpredictability for an artisan to achieve optimal *in vivo* efficiency for targeting of the inventive recombinant nucleic acid to a chosen cell type, optimal *in vivo* efficiency for gene transfer of the inventive recombinant nucleic acid, and optimal *in vivo* efficiency to achieve effective expression levels of the eNOS polypeptide, for long enough periods to effect the therapeutic results.

With respect to the genus of eNOS polypeptides contemplated to be encoded by the inventive nucleic acid to effect the therapeutic treatments, the art recognizes that protein chemistry is probably one of the most unpredictable areas of biotechnology. Applicant contemplates a genus of mutations in human eNOS that result in i) an increase or decrease of the eNOS polypeptide (Claim 12), ii) an increased binding affinity for calmodulin of the mutant eNOS polypeptide (Claim 13), iii) a decrease in Ca2+-calmodulin dependent stimulation of the mutant eNOS polypeptide (Claim 14), and iv) an increased eNOS activity, as measured by the generation of nitric oxide (Claims 15-16) and reductase activity (Claims 15, 17). In the instant case, the art recognizes that dephosphorylation of Thr495 facilitates the binding of calmodulin to the CaM-binding domain of eNOS, and thus is a prerequisite for maximal NO production (Lenasi et al, Cardiovascular Research 59: 844-853, 2003; pg 851, column 2, ¶3, topic sentence). Furthermore, the art recognizes that phosphorylation of Ser1177, or mutation of Ser1177 to Aspartate (S1177D) promotes NO synthesis (Fulton et al, pg 599, Figure 3; *of record in IDS). However, the art is silent with respect to the functional activity of an eNOS polypeptide that is double or triple mutant for Thr495 and Ser1177 and Gly2 substitutions.

While it is known that many amino acid substitutions are possible in any given protein, the position within the protein's sequence where such amino acid substitutions can be made with reasonable expectation of success are limited. Certain positions in the sequence are critical to the three-dimensional structure/function relationship, and these regions can tolerate only conservative substitutions or no substitutions. Residues that are directly involved in protein functions such as binding will certainly be among the most conserved (Bowie et al, Science 247:1306-1310, 1990; pg 1306, column 2). Reasonable correlation must exist between the scope of the claims and scope of enablement set forth, and it cannot be predicted from the disclosure how to use all the eNOS mutants and the homologs thereof.

The art recognizes that the relationship between the amino acid sequence of a protein (polypeptide) and its tertiary structure (i.e. its binding activity) are not well understood and are not predictable (see Ngo et al., in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz, et al., (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495). There is no recognition in the art that sequence with identity predicts biological function. It is known in the art that even single amino acid changes or differences in a protein's amino acid sequence can have dramatic effects on the protein's function. For example, conservative replacement of a single "Lysine" reside at position 118 of acidic fibroblast growth factor (aFGF) by "Glutamic acid" led to the substantial loss of heparin binding, receptor binding and biological activity of the protein (Burgess et al, J of Cell Bio. 111:2129-2138, 1990). In transforming growth factor alpha (TGF-α) replacement of Aspartic acid at position 47 with Alanine or Asparagine did not affect biological activity while replacement with Serine or Glutamic acid sharply reduced the biological activity of the mitogen (Lazar et al, Molecular and Cellular Biology 8:1247-1252, 1988). These references demonstrate that even a single amino acid substitution or what appears to be an inconsequential chemical modification will often dramatically affect the biological activity and characteristic of a protein. Furthermore, the specification fails to teach what deletions, truncations, substitutions and mutations of the disclosed sequence can be tolerated that will allow the protein to function as claimed.

With respect to the animal model used in the instant application, the art reasonably questions the validity of such animal models to adequately reflect real world ischemic

conditions. Messina et al teaches "The evaluation of potential molecular therapies has been hampered by the lack of suitable experimental models. For example, quantitative assessment of the benefit of angiogenic therapy is complicated by the spontaneous neovascularization and collateral formation that characterizes small animal models of hindlimb ischemia. It is therefore unclear how these animal models of what is actually acute ischemia relate to the clinical scenario in which a patient's limb has had decreased blood flow for weeks to months, and has by definition clearly failed to spontaneously mount an adequate angiogenic response." (pg 286, column 2, ¶2).

With respect to protein therapy, the art teaches that although numerous protein delivery methods are describe in the art, only some of these methods have been tested *in vivo* and very few have human clinical data to demonstrate the feasibility of the method (Cleland et al, April, Current Opinion in Biotechnology 12: 212-219, 2001). Several critical issues require evaluation to move a method from basic research to clinical practice, and include: providing significant levels of protein at the diseased site, avoiding unwanted side effects and toxicity if high systemic levels are necessary to achieve local effect in target tissue, the impact of manufacturing required for the delivery method on the integrity of the protein, the integrity of the protein after delivery to the patient, as administration of degraded protein may result in unwanted side effects or loss of potency, and the bioavailability of the protein post-administration (page 213, columns 1-2). Furthermore, preclinical animal models do not always predict the behavior of the delivery system in humans due to biological or genetic differences. Thus, the art recognizes considerable uncertainty in the ability to effectively administer a therapeutic protein to any diseased target site by any of all possible delivery means proposed in the art to effect the desired therapeutic result.

In the instant case, the art teaches that FGF protein administration to patients with ischemic disease failed to demonstrate an overall improvement in the size of the ischemic territory, and thus the idea of FGF protein therapy has failed to translate into clinical benefit (Khurana et al, pg 1211-1212, joining ¶; * of record in IDS). Furthermore, the art recognizes uncertainty regarding whether FGF administration should be targeted to the ischemic, peri-ischemic or non-ischemic zones (pg 1212, column 1, ¶3, lines 7-9). The art also recognizes that the optimal biological FGF protein therapy has yet to identified, as FGF induces proliferation

and migration of vascular smooth muscle cells, which could trigger restenosis, and thus be detrimental in blood vessels (Messina et al, pg 286, column 2, lines 2-7; * of record in IDS).

Thus, one of ordinary skill in the art reasonably recognizes the unpredictability in the enzymatic activity of the contemplated double-mutant eNOS polypeptide, the ability to effectively target the inventive polynucleotide to the desired cell types, the ability to administer an effective amount of angiogenic protein factors before, during or after administration of the polynucleotide, and the validity of the instant mouse and rat models disclosed in the specification to adequately represent the real world conditions of clinical ischemic disease. Furthermore, the art is silent with respect to methods of treating chronic limb ischemia wherein both a polynucleotide encoding a therapeutic polypeptide and an angiogenic polypeptide are administered to the patient, wherein the angiogenic polypeptide is administered before, during or after the administration of the polynucleotide.

The Existence of Working Examples and The Amount of Direction Provided by the Inventor

The instant invention is drawn to a method of administering a polynucleotide encoding a human eNOS polypeptide comprising two amino acid mutations, in which the first mutation corresponding to Threonine 495 of SEQ ID NO:1 is mutated to Alanine, Valine, Leucine or Isoleucine, the second mutation corresponding to Serine 1177 of SEQ ID NO:1 is mutated to Aspartate, and the third mutation corresponding to Gly 2 of SEQ ID NO:1 is mutated to Alanine. Applicant further contemplates the inventive method to comprise the administration of angiogenic proteins before, during or after the administration of the inventive eNOS polynucleotide. However, the breadth of the instantly claimed invention is mere contemplation, as the specification provides no working examples of the administration of a polynucleotide encoding an eNOS double- or triple-mutant polypeptide with or without the co-administration of an angiogenic factor with said polynucleotide. Rather, the instant working examples disclose the intra-muscular injection of a polynucleotide encoding the single mutant S1177D eNOS polypeptide. Furthermore, Applicant claims the invention to specifically target endothelial and bone marrow-derived cells. However, as discussed above, the art recognizes that numerous and diverse cell types exist within, and are derived from, the bone marrow, and the specification fails

to provide sufficient guidance to an artisan how to effectively deliver the inventive polynucleotide(s) to any and all of these art-recognized bone marrow-derived and endothelial cell types. There is no demonstration that the intramuscular injection of adenovirus particles encoding the S1177D eNOS polypeptide specifically targeted the endothelial and bone marrow-derived cells.

Applicant contemplates that mutations in human eNOS embraced by the invention will result in i) an increase or decrease of the eNOS polypeptide (Claim 12), ii) an increased binding affinity for calmodulin of the mutant eNOS polypeptide (Claim 13), iii) a decrease in Ca2+-calmodulin dependent stimulation of the mutant eNOS polypeptide (Claim 14), and iv) an increased eNOS activity, as measured by the generation of nitric oxide (Claims 15-16) and reductase activity (Claims 15, 17). The specification teaches a method of making the human eNOS mutants having mutations at positions Thr495, Ser1177 and Gly2, and assays for detection and measurement of the activity of said eNOS mutants (pgs 20-21, Figure 2). However, the specification does not disclose eNOS mutants having mutations at any other positions. It is noted that the *in vivo* working examples disclose the use of eNOS S1177D, but not of eNOS T495X alone or in combination with S1177D or Gly2. Furthermore, Figure 2 does not provide the *in vitro* NO production of the S1177D eNOS single mutant or of any mutant eNOS polypeptide comprising the recited Gly2 mutation, prohibiting a necessary comparison with the inventive eNOS polypeptides. Thus, an artisan cannot compare between the activities of the inventive eNOS polypeptides and correlate their successfulness in the disclosed rodent surgical models.

While one of ordinary skill in the art can theoretically produce all of these eNOS polypeptide mutants with art known techniques such as site-directed mutagenesis it would still be burdensome to one of ordinary skill in the art to produce all of these different mutations and thereafter determine their activity. It is art-recognized that certain residues are shown to particularly important to the biological or structural properties of a protein or peptide, e.g., residues in active sites and such residues may not be generally be exchanged. Skolnick et al teach that sequence-based methods for function prediction are inadequate and knowing a protein's structure does not tell one its function (Skolnick et al, Trends in Biotech. 18: 34-39, 2000; see abstract, in particular). Given the unlimited number of undisclosed eNOS mutants,

there are insufficient working examples demonstrating that the undisclosed mutants can still function as wild type eNOS or have, for example, increased enzyme activity. Moreover, it is not clear what criteria would be used in deciding which amino acids and how many of them would and could be substituted in the wild type proteins. Without such guidance, the changes which can be made in the protein structure and still maintain activity is unpredictable and the experimentation left to those skilled in the art is unnecessarily and improperly extensive and undue. See *Amgen Inc. V. Chugai Pharmaceutical Co. Lts.*, 18 USPQ2d 1016 and *Ex parte Forman*, 230 USPQ 546 (BPAI 1986). For these reasons, it would require undue experimentation of one skilled in the art to practice the claimed invention. See page 1338, footnote 7 of *Ex parte Agarwal*, 23 USPQ2d 1334 (PTO Bd. Pat App. & Inter. 1992).

The Quantity of Any Necessary Experimentation to Make or Use the Invention

Thus given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of the art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the lack of working examples in the specification which address the issue of making and using all the claimed eNOS mutants and homologs, and the negative teachings in the prior art balanced only against the high skill level in the art, it is the position of the instant Examiner that it would require undue experimentation for one of ordinary skill in the art to perform the method of the claim as broadly written.

With respect to the genus of mutations resulting in an eNOS polypeptide having increased stability and/or biological activity, while methods of isolating or generating variants of a polypeptide were known in the art at the time of the invention, it was not routine in the art to screen - by a trial and error process - for all polypeptide variants having a substantial number of modifications as encompassed by the claims for those polypeptides having the desired activity/utility. The quantity of experimentation in this area is extremely large since there is significant variability in the structure and effects of the eNOS polypeptide mutants and the homologues thereof. Moreover, it would require significant study to determine which of the eNOS polypeptide mutants in fact have the claimed function (e.g. increased binding affinity for calmodulin, or increased eNOS activity). The identification and characterization of each of these eNOS mutants and homologues would be inventive, unpredictable, and difficult in itself,

requiring years of inventive effort with no guarantee of success in doing so. The reference of Guo et al (Proc Natl Acad Sci 101(25):9205-9210, 2004) teaches a study suggesting that the percentage of variants having multiple substitutions that maintain activity appears to be exponentially related by the simple formula: $(.66)^x$ X 100% (where x is the number of mutations introduced). Looking at the most limited of the variant claims (Claim 37) wherein the polypeptide is limited to a polypeptide having at least 95% identity to SEQ ID NO:l, the polypeptide can have 5% of the amino acids altered. Thus, up to 60 amino acids within the 1200 amino acids of SEQ ID NO:I can be simultaneously mutated. According to Guo et al., only $(.66)^{60}$ X 100% or 1.5 X 10^{-11}% of random mutants having 95% identity to SEQ ID NO:1 would be active. Thus, a significant number of variants must be screened in order to isolate those variants of SEQ ID NO:1 having the desired eNOS activity recited in Claims 12-17. The art clearly does not typically engage in the screening of such a large number of variants to isolate those relatively few variants that would have the desired activity/utility. That screening this number of variants is not routinely practiced in the art is evidenced by Hult and Berglund (Curr. Opin. Biotechnol. 14:395-400, 2003), which teaches that recent attempts to randomly obtain variants of a given polypeptide included screening of "6000 transformants" (p. 396, left column, top) or 3.4 x 10^7 variants (p. 396, left column, bottom).

In conclusion, the specification fails to provide any guidance as to how an artisan would have dealt with the art-recognized limitations of the claimed method commensurate with the scope of the claimed invention and therefore, limiting the claimed invention to a method of treating critical limb ischemia (CLI) comprising administering by intramuscular injection to a patient in need of treatment an effective amount of a recombinant adenovirus comprising a polynucleotide encoding a mutant mammalian endothelial nitrogen oxide synthase (eNOS) polypeptide, wherein the mutation consists of the amino acid residue corresponding to amino acid residue 1177 of SEQ ID NO:1 being substituted to Aspartate, is proper.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the Applicant regards as his invention.

9. **Claims 12-21 are rejected under 35 U.S.C. 112, second paragraph**, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Regarding Claims 12-17, the phrase "as compared to a reference eNOS polypeptide" renders the claim(s) indefinite because the claim(s) include(s) elements not actually disclosed (those encompassed by "a reference eNOS polypeptide"), thereby rendering the scope of the claim(s) unascertainable. See MPEP § 2173.05(d).

Claims 18-19 recite alternative eNOS reference polypeptides, wherein the mutant and non-mutant eNOS polypeptides have different enzymatic activities. Furthermore, the phrase "derived from" broadens the scope of reference eNOS polypeptides to include those polypeptides which do not actually have eNOS activity. Thus, an artisan is unable to ascertain, *a priori*, what amount of eNOS activity is encompassed by the claimed invention.

Claims 18-19 are indefinite for reciting the phrase "is derived from SEQ ID NO: 1" as the exact meaning of the word "derived" is not known. The term "derived" is not one which has a universally accepted meaning in the art nor is it one which has been adequately described in the specification. The primary deficiency in the use of this phrase is the absence of an ascertainable meaning for said phrase. It is unclear how the SEQ ID NO:1 is to be derivatized.

Claims 20-21 recite the term "substantially", which is a relative term and renders the claim indefinite. The term "substantially" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Furthermore, the claims are dependent on Claim 4, wherein the eNOS polypeptide comprises a mutation, yet recites the non-mutant eNOS having the amino acid sequence of SEQ ID NO:1 recited in Claim 1. As presently recited, it is unclear if the substantial homologies are required of the non-mutant SEQ ID NO:1 or a mutant SEQ ID NO:1.

Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

10. **Claims 1-2, 4, 6, 10, 12-13, 15-18, 20-26, 32, 34-36 and 39 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Smith et al (* of record in IDS) and Fulton et al (* of record in IDS).

The claims are drawn to a method of treating critical limb ischemia comprising administering to a patient in need of treatment an effective amount of a polynucleotide encoding a mammalian endothelial nitrogen oxide synthase (eNOS) polypeptide.

Smith et al teach a method of administering to a rat hindlimb ischemic model a polynucleotide encoding a human wildtype eNOS polypeptide (pg 1280, column 2, "Femoral Artery Ligation"; pg 1281, column 2, "Time Course of Human eNOS Expression"). The polynucleotide encoding the human eNOS was contained in an adenoviral vector operably linked to a cytomegalovirus (CMV) promoter (pg 1279, column 2, lines 14-17; pg 1280, column 1,

"Construction of eNOS Adenovirus").The adenoviral vector is administered by intramuscular injection, wherein eNOS activity is thus modulated in the cells of said patient (pg 1281-1284, Results).

Smith et al do not teach a method of treating critical limb ischemia comprising the administration of an adenovirus encoding a mutant mammalian eNOS polypeptide, wherein the mutation consists of the amino acid residue corresponding to amino acid residue 1177 of SEQ ID NO:1 being substituted to Aspartate. However, at the time of the invention, Fulton et al taught that the substitution of the amino acid residue Serine corresponding to amino acid residue 1177 of SEQ ID NO:1 to Aspartate (S1177D) results in a gain-of-function enzyme (pg 599, Figure 3 and legend). Smith et al note that the S1179 of the instant eNOS corresponds to S1177 of human eNOS (pg 599, column 2, lines 1-3), and thus also corresponds to amino acid residue 1177 of SEQ ID NO:1. Fulton et al teach that phosphorylation of S1177 is functionally important for NO release, and thus permits activated calmodulin-binding at lower calcium concentrations (pg 600, column 1, line 22; column 2, lines 20-22). Furthermore, Fulton et al suggest that regulation of such phosphorylation may provide a novel therapeutic target for the design of drugs aimed at improving endothelial function in cardiovascular disease associated with dysfunction in the synthesis or biological activity of NO (pg 600, column 2, conclusory sentence).

It would have been obvious to one of ordinary skill in the art to modify the adenoviral vector encoding the human eNOS polypeptide of Smith et al with the S1177D mutant eNOS polypeptide taught by Fulton et al with a reasonable chance of success because Fulton et al teach that the S1177D mutation results in a enzyme with greater activity.

An artisan would have been motivated to substitute wildtype eNOS for the S1177D eNOS because wildtype eNOS requires post-translational modifications, specifically phosphorylation of particular amino acid residues including 1177, that regulates and limits the activity of the normal enzyme; whereas, the S1177D mutation mimics the activating post-translational modification, obviates the requirement for regulated activation, decreases sensitivity to NOS inhibitors, and thus results in an enzyme *a priori* with as much activity as a fully activated wildtype eNOS polypeptide. Furthermore, Fulton et al suggest that drugs that promote the phosphorylation of eNOS, and thereby activating eNOS, would be of therapeutic value. The instant specification discloses that the S1177D eNOS mutation can be more efficacious than

wild-type eNOS (pg 11, line 21). Given that S1177D results in a gain-of-function eNOS enzyme, it would be more efficient and advantageous to use the existing mutation rather than having the artisan expend resources to identify a drug with that will promote the same enzymatic activity, though more indirectly, as the instant mutation.

Thus, Claims 1-2, 4, 6, 10, 12-13, 15-18, 20-26, 32, 34-36 and 39 are *prima facie* obvious.

11. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Kevin K. Hill, Ph.D. whose telephone number is 571-272-8036. The Examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Joseph T. Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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